Molecular Order in Spider Major Ampullate Silk (Dragline): Effects of Spinning Rate and Post-Spin Drawing

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ABSTRACT: Optical birefringence measurements are used to characterize how the molecular order of spider (*Nephila clavipes*) major ampullate silk is affected by linear spinning rate, by the extent of post-spin drawing, and by post-spin drawing rate. Results are interpreted qualitatively in terms of a simple microstructural model, in which birefringence depends on both the overall degree of molecular orientation and the extent to which crystalline regions are present. In contrast to the behavior of conventional, synthetic polymers, birefringence is found to be an unreliable predictor of tensile stiffness: microstructural changes that lead to increased birefringence may leave stiffness unchanged or, in some cases, lower than before. It is unlikely that economic processing of silk-like polymers into fiber that exhibits biomimetic tensile properties can be achieved with spinning followed by drawing, or with a single spinning step. Instead, spinning followed by thermochemical treatment under load may be needed to obtain the critical combination of molecular orientation and crystallinity in commercially satisfactory time scales. © 1999 John Wiley & Sons, Inc. J Appl Polym Sci 72: 895–903, 1999

Key words: birefringence; drawing; molecular order; silk; spinning; stiffness

INTRODUCTION

Spider major ampullate silk (MAS) exhibits an impressive combination of tensile strength, stiffness, and toughness.¹ As a result, efforts have been made by several investigators to characterize the hierarchical molecular order in this material²⁻¹⁰ and to relate molecular order to mechanical properties.^{11,12} Studies have focused on the microstructure of silk spun at rates comparable with those used in web building ($\sim 1 \text{ cm s}^{-1}$). Little attention has been paid to the underlying physical process variables, such as changing the linear spinning rate or subjecting the fiber to a post-spin draw in the solid state. Such knowledge

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would be useful in attempts to develop biomimetic fibers that reproduce or perhaps even exceed the outstanding tensile properties of natural silk.

Conventional manufacture of synthetic fibers depends on one or more post-spin drawing procedures for introducing most of the molecular alignment preserved in the product.¹³ In contrast, silkspinning organisms make do with a single processing step, because the conditions under which they have to produce fiber preclude post-spin drawing. Spinning rates for a given type of silk can be highly variable, fluctuating between zero and several centimeters per second, while consistently delivering a material with dependable properties. Spiders hook a leg around the dragline to pull it out of the spinneret during web building, or they use their weight when spinning dragline to escape a predator, or they may simply depend on air currents to catch the extruded silk when spinning dragline for use in "ballooning."¹⁴ Silk-

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worms have to keep moving to pull their cocoon silk from the spinneret; again, the spinning rate fluctuates, and there is no post-spinning draw. It is apparent, therefore, that the processing conditions used to optimize molecular order and mechanical properties in typical synthetic polymer fibers may not be similarly applicable in the context of silk-like polymers.

EXPERIMENTAL

Collecting Spider MAS

MAS was reeled from mature Nephila clavipes spiders at forced rates of 0.5 cm s⁻¹ and 10 cm s⁻¹. These represent extremes in the range of spinning rates at which functional MAS fiber is produced naturally. The low rate is typical for web building, whereas the high rate pertains to controlled vertical descents made by spiders eluding predators. The procedure for "silking" spiders was adapted^{15,16} from a widely cited method.¹⁷ For the present experiments, single major ampullate fiber was collected from the spider onto a rotating and simultaneously translating Teflon mandrel.

Lengths of silk were then transferred from the mandrel onto pieces of card for storage, maintaining some slack between the card and the mandrel to avoid stretching the fiber. Black card was used to provide a contrasting background against which the silk could most easily be seen. Card dimensions were $\sim 4 \text{ cm} \times 10 \text{ cm}$. Edges were masked with adhesive tape to prevent the silk from snagging.) The fiber was wound parallel to the long direction of the card, taking care to leave a space between each turn. Each card could hold ~ 2 m of silk. After the transfer was completed, two small tabs of tape were placed on the silk fiber leading from the mandrel, and the silk was cut between them. In this manner, the ends of the cut silk could be located easily. The tab on the silk connected to the card was taped to the card, whereas the tab on the silk connected to the mandrel was used to start another card. The cards were stored in air-tight containers in a dark location.

Post-spin Draw

Again, taking care not to stretch the fiber, lengths of MAS were transferred from the storage cards to sample holders prepared from graph paper (Fig-



Figure 1 Steps in preparing MAS samples for postspin drawing.

ure 1). The fiber was secured across a 5 cm "window" cut in the graph paper, using first doublesided adhesive tape and then cyanoacrylate superglue to prevent slippage when the samples were strained. Lines on the graph paper bordering the "window" served as guides in orienting the fiber. The 5-cm gauge length always contained only material that had been stored against the flat sides of the cards; 5 cm is consistent with studies^{11,18} that characterized the tensile mechanical properties of spider MAS. The doublesided tape and superglue were covered with insulating tape to provide a rubberized surface for the mechanical grips of the tensometer to hold.

Single-fiber samples and their holders were mounted in an Instron model 1026. A straight



Figure 2 Steps in retrieving strained MAS for birefringence characterization by light microscopy.

vertical edge and oblique lighting were used to ensure that fibers were oriented vertically, after which the sides of the sample holder were cut away [Figure 1(d)]. Samples were strained at controlled, constant rates of either 1 mm min⁻¹ $(3.3 \times 10^{-4} \text{ s}^{-1})$ or 100 mm min⁻¹ $(3.3 \times 10^{-2} \text{ s}^{-1})$ to engineering (nominal) strains of 5%, 10%, 15%, or 20%. Because many samples broke at between 15% and 20% strain, we only quote behavior for strains up to 15% in our results. Laboratory temperature and humidity remained approximately constant at $21 \pm 1^{\circ}$ C and $62 \pm 3\%$, respectively.

Molecular Order

Sections of the strained samples were retrieved onto standard glass microscope slides. Apart from ensuring that samples are not lost, the method of transfer (Figure 2) keeps samples straight, which facilitates characterization of molecular order by light microscopy. Also, the samples as held are unable to undergo macroscopic retraction, which limits the rate at which molecular alignment introduced by drawing can subsequently relax.

Optical retardation of strained samples was measured by the de Sénarmont method¹⁹ on an Ultraphot II light microscope (Carl Zeiss, Oberkochen/Württemberg, Germany). The de Sénarmont method is especially well suited to measuring small retardations and small changes in retardation: even though the silk samples exhibit a high degree of molecular anisotropy, the retardation values are low because the samples are thin. Measurements were performed within 10 min of straining.

To convert optical retardation into birefringence, it is necessary to divide by the sample thickness. Thickness was measured with a calibrated eyepiece graticule. Because the cross-sectional geometry of MAS can vary along even short lengths of fiber,²⁰ all birefringence results quoted herein are averages. At each of three locations along the sample, the retardation and diameter were measured three times. These data were used to calculate nine values of birefringence, from which the average was obtained.

Controls

We must consider the possibility that molecular alignment in as-collected N. clavipes MAS might relax, in which case the timing of experiments conducted on this material would become important. Control experiments were therefore performed (C. Viney and K. Kerkam, Department of Materials Science and Engineering, University of Washington, Seattle, WA) to monitor the birefringence of as-collected fiber over extended periods of time. The collection procedure was similar to that described herein, but only short lengths of fiber were reeled, and these were transferred directly onto glass microscope slides for retardation measurement. Initial retardation measurements could be made within 5 min of the silk being collected. A Berek compensator (less accurate than the de Sénarmont method, but requiring less time to obtain readings²¹) was used in these controls.

Finally, we also performed controls to determine whether the birefringence changes with time in samples that have been subjected to postspin drawing. Samples strained to 15% and used in the main series of experiments, were retained on their glass slides, and their birefringence was measured again 1 month later.

RESULTS AND DISCUSSION

Background to Interpretation

The birefringence exhibited by silk fiber cannot be linked to a single microstructural variable. At least two types of microstructural change are known to occur when *N. clavipes* MAS is strained past its yield point. There is an increase in the overall degree of molecular alignment.¹⁰ Also, Xray diffraction suggests that the size of the (most highly) ordered regions in MAS is reduced by strain¹⁰; we would expect the internally less wellordered nonperiodic lattice (NPL) crystallites^{7,8} to be even more susceptible to degradation when deformed.

An increase in the degree of polymer chain extension and orientation throughout the microstructure will lead to a higher value of birefringence, as the distribution of polarizable bonds becomes more anisotropic. Succinctly: increased *orientational* order leads to increased birefringence.

In contrast, if chains are parallel and able to develop lateral registry, the resulting crystals should have a *lower* birefringence than a similar volume of aligned but noncrystalline polymer. When β -sheet-ordered regions (either conventional crystals or NPL crystals) develop in solidifying silk, the formation of interchain hydrogen bonds within sheets and of van der Waals or hydrogen bonds between sheets must be accompanied by increased polarizability in both these transverse directions. Given that the strongest bonding is along the chains, that will be the direction of highest polarizability. However, the difference between the longitudinal and transverse polarizabilities will be smaller in the β -sheet regions than in material consisting of aligned, uncrystallized chains. Succinctly, increased positional order leads to decreased birefringence.

Therefore, both the sharpening of molecular alignment and the degradation of ordered regions during fiber drawing will contribute to increased birefringence. The same two microstructural changes addressed in this discussion have opposing effects on fiber stiffness. Increased orientational order will enhance the tensile modulus, whereas the concomitant lower volume fraction of ordered regions will diminish it. Experimental evidence^{11,22} that the stiffness of silk is not sensitive to fiber processing conditions will be referred to in the discussion herein.



Figure 3 Plot of birefringence *versus* time for MAS reeled at 0.5 cm s^{-1} . Birefringence readings were taken at $\sim 5 \text{ min}$, 1 h, 1 day, 29 days, 48 days, and 733 days.

Initial Birefringence of As-Collected MAS

Figure 3 shows the results of a typical control experiment to monitor the birefringence of N. clavipes MAS collected at 0.5 cm s⁻¹; in this case, measurements were taken over an \sim 2-year period. The variation in birefringence is negligible; any relaxation of molecular orientation or other significant microstructural change would have had to occur within the first few minutes after silking. We can therefore be confident that the results of our experiments are not sensitive to the length of time for which the silk is stored prior to post-spin drawing.

Figure 4(a,b) shows birefringence as a function of post-spin drawing at engineering strain rates of 0.00033 s^{-1} and 0.033 s^{-1} , for MAS collected at 0.5 cm s^{-1} . The silk used for compiling the two parts of Figure 4 was obtained from different spiders. Significantly different values of initial birefringence are recorded: 0.038 and 0.047 in Figure 4(a,b, respectively). This difference does not imply a large random variability in the birefringence of silk collected at a fixed rate, but is principally a systematic variation that is linked to the initial thickness of the as-spun silk: $5.0(3) \mu m$ and $3.7(7) \ \mu m$ for the specimens characterized in Figure 4(a,b, respectively). Parentheses around the last significant digit in each thickness value are a reminder that it represents a level of accuracy obtained by averaging and not by direct measurement. Spinning at a nominally fixed rate causes thinner fiber to experience a larger average shear stress²³ and therefore to develop a greater degree of molecular alignment, leading to a higher value of birefringence. In experiments where MAS collected at increasing rates (0.5 cm s⁻¹, 1.0 cm s⁻¹, and 2.0 cm s⁻¹) exhibited fortuitously similar thickness, the corresponding increase in birefringence was marked (0.025 \pm 0.001, 0.033 \pm 0.001, and 0.041 \pm 0.001).²⁴ Supporting results have been found over a wider range of silking rates, using two different methods of birefringence characterization.¹⁶

Figure 5(a,b) shows the equivalent information for MAS collected at 10 cm s⁻¹, again from two different spiders. These two samples coincidentally have an identical thickness of $3.1(4) \mu$ m; it is therefore not surprising that their respective values of birefringence (0.056 and 0.055) differ by no more than the error in measurement.

It is not possible to dependably predetermine the initial thickness of as-collected silk. Even though all spiders used in this study were mature, at a comparable stage in their development, they produced MAS of widely differing diameters due to different spinneret sizes. Also, spinnerets are active devices, under the control of the spider. Although spiders can be anesthetised before silk is collected, it has not been proved that the physiology of supramolecular self-assembly remains unaffected, and anesthesia would, in any case, not eliminate the effects of intrinsic variability in spinneret diameter. We therefore cannot straightforwardly use absolute values of birefringence to characterize the dependence of molecular order on collection rate, and the rate and extent of subsequent drawing. Instead, we have to concentrate on the magnitude of the *change* in birefringence when the as-collected silk is drawn.

Post-spin Drawing

For MAS collected at 10 cm s⁻¹ [Figure 5(a,b)], birefringence is markedly more sensitive to strain than in the case of material collected at 0.5 cm s⁻¹ [Figure 4(a,b)]. This observation holds at both of the post-spin strain rates used. The birefringence–strain relationship is insensitive to strain *rate* in the MAS collected at 0.5 cm s⁻¹; but, birefringence at a given strain is consistently and significantly higher for the 3.3×10^{-2} s⁻¹ strain rate if the fiber was reeled at 10 cm s⁻¹.

Spinning conditions therefore significantly affect the level of microstructural anisotropy that can be induced in silk fiber by subsequent drawing. From electron microscopy,⁸ it is known that the volume fraction and especially the size of three-dimensionally ordered regions in MAS decreases as the collection/solidification rate in-

creases. The ability of the ordered regions to form while fiber is spun depends on the extent to which statistical matches can develop between adjacent chains, which in turn depends on the opportunity for chain mobility. As microstructural pinning by ordered regions is reduced, it becomes increasingly possible for subsequent drawing in the solid state to enhance molecular alignment. A similar principle lies behind the processes of superdrawing and gel drawing to produce high modulus polymer fibers,^{25,26} where superlative degrees of alignment are achieved by limiting the initial incidence of microstructural pinning. The overall level of pinning will also affect susceptibility to degradation of the pinning sites themselves. Commercial solution spinning rates typically¹³ lie in the range of 1300-1500 cm s⁻¹, and we expect these values to apply as well to the spinning of MAS analogs produced in bulk quantities *via* biotechnological routes. Such rates are two to three orders of magnitude greater than those that pertain to our present work. Extrapolating our results suggests that drawing fiber spun at commercially economic rates should yield highly anisotropic material. However, as will become apparent in the next section, drawing-induced anisotropy relaxes more readily in fibers where the microstructural pinning is less effective; also, enhanced anisotropy does not necessarily equate to enhanced tensile stiffness.

We now consider the effect of post-spin draw rate. As draw rate (which in all cases considered is large, compared with microstructural relaxation rates) increases, so will the energy temporarily stored in the deformed microstructure. This energy provides a driving force for solid-state molecular mobility, increasing molecular alignment and degrading pinning centers. The sensitivity of microstructural change to draw rate will be greatest when pinning is least effective (i.e., for silk acquired at the higher collection rate).

The degradation of ordered regions, regardless of their degree of internal perfection, can be expected to result in a fall in fiber stiffness. On the other hand, increased chain alignment in amorphous regions, as well as enhanced relative alignment of the remaining ordered regions, should produce a rise in stiffness. This combination of events leads to the interesting prospect of fiber modulus remaining approximately independent of macroscopic plastic deformation, while fiber birefringence is an increasing function of such deformation. It has been observed in the case of *Bombyx mori* (silkworm) silk that, even following



Figure 4 Dependence of birefringence on strain and strain rate, for MAS reeled at 0.5 cm s^{-1} from two different spiders. "Nails" indicate the positive half of error bars.

deformation close to the failure strain, the elastic modulus is the same as it was before deformation.²² N. clavipes MAS reeled at 1 cm s⁻¹ exhibits tensile properties (including stiffness) that are not statistically distinguishable from fiber reeled at 10 cm s⁻¹ (see ref. 11). In fact, earlier evidence suggests that this observation holds over an even wider range of silking rates.¹⁸ Yet, the birefringence obtained from MAS fibers of similar thickness varies significantly with collection rate.²⁴

Permanence of the Birefringence Change Induced by Post-spin Drawing

No changes in optical retardation or fiber thickness were detected during the few minutes needed to characterize birefringence immediately after post-spin drawing. Constraining the length of samples used for microscopy will help to retard the rate of any microstructural relaxation. However, birefringence was observed to have decreased in those samples (all drawn to 15% strain) that were characterized again 1 month later. Figures 6 and 7, respectively, show data obtained from the samples characterized in Figures 4 and 5. The two samples initially reeled at 0.5 cm s⁻¹ (Figure 6) show closely similar relaxation behavior, as do the two samples collected at 10 cm s⁻¹ (Figure 7).

We do not know how long it takes for the birefringence of the drawn fibers to decrease to the values measured 1 month later, but no further decrease in birefringence is detected with the passage of time, even after several additional months. The birefringence values in the rightmost pair of columns of Figures 6 and 7 therefore relate to microstructures that have attained at least metastable equilibrium at room temperature.

We have already referenced the fact that X-ray diffraction reveals a sharpening of orientational molecular order in response to post-spin straining.¹⁰ At the same time, we also noted the evidence that strain reduces the size of the (most



Figure 5 Dependence of birefringence on strain and strain rate, for MAS reeled at 10 cm s^{-1} from two different spiders. "Nails" indicate the positive half of error bars.



Figure 6 Birefringence as a function of mechanical history, for MAS reeled at 0.5 cm s^{-1} from two different spiders. Data labeled "As spun" and "+15% strain" are repeated from Figure 4. "Nails" indicate the positive half of error bars.

crystalline) ordered regions in MAS, and commented that such degradation would be even easier in the case of the internally less well-ordered NPL crystallites identified in silk.⁸ We cannot envisage a mechanism of solid-state molecular translation whereby the degraded crystals (or NPL crystals) would be driven to reform after straining. As a first approximation, we therefore assign the permanent birefringence change to the irreversible degradation of microstructural pinning centers. The recoverable component of birefringence change induced by drawing is assigned to changes in molecular orientation. In this context, weaker pinning is seen to allow a greater permanent increase in birefringence ($\sim 15\%$ in silk spun at 0.5 cm s $^{-1}$ versus \sim 20% in silk spun at 10 cm s⁻¹, in both cases after 15% strain); meanwhile, there is no statistically significant dependence on strain rate. The recoverable component of birefringence increase is also greater for the higher spinning rate, but does exhibit strain rate dependence. The implication that molecular orientation changes are more sensitive than degradation of pinning centers, in regard to dependence on strain rate, is unsurprising: the former response does not *require* intermolecular bonds to be broken.

From the point of view of processing, post-spin plastic strain enhances microstructural and therefore optical anisotropy, some of which is preserved. However, the increased anisotropy does not necessarily herald an increase in tensile modulus. In fact, if the permanent component of birefringence increase is principally due to degradation of ordered regions, there may even be a concomitant *decrease* in stiffness that would become more evident with the relaxation of strain-induced molecular alignment. In contrast to the methods conventionally used to maximize stiffness in synthetic polymers, our observations sug-



Figure 7 Birefringence as a function of mechanical history, for MAS reeled at 10 cm s^{-1} from two different spiders. Data labeled "As spun" and "+15% strain" are repeated from Figure 5. "Nails" indicate the positive half of error bars.

gest that silk analogs should not be post-spin drawn. The maximum stiffness will be obtained by selecting a spinning rate that establishes an optimum balance between overall molecular alignment and internally ordered reinforcing entities in the microstructure. Measurement of thermal expansion, which does not require control or measurement of sample diameter, show that this optimum is achieved at spinning rates close to 2 cm s⁻¹ (see ref. 27). It has recently been shown²⁸ that B. mori silk can be solubilized, spun into fiber, and then hand-drawn after lengthy and continuous immersion in coagulant (swelling agent) to generate molecular order resembling that found in naturally spun material. It seems unlikely, however, that centimeter-per-second rates can form the basis of an economically viable process. A more plausible approach might involve spinning at typical commercial rates, followed by providing a combination of temperature, chemical environment, and stress that preserve chain alignment while allowing molecules to form reinforcing crystalline or NPL regions.

SUMMARY AND CONCLUSIONS

- 1. The optical anisotropy of as-spun *N. clavipes* MAS remains stable with time. No significant microstructural changes occur in the fiber after spinning.
- 2. The rate at which the silk is spun or reeled affects several aspects of its microstructural and optical anisotropy:
 - a. Initial birefringence is an increasing function of spinning rate, if the fiber thickness is constant.
 - b. The rate at which birefringence increases with post-spin strain is greater in fiber spun at 10 cm s⁻¹, compared with fiber spun at 0.5 cm s⁻¹.
 - c. The rate at which birefringence increases with post-spin strain is sensitive to the strain *rate* in fiber spun at 10 cm s⁻¹, but not in fiber spun at 0.5 cm s⁻¹.
 - d. The irrecoverable component of birefringence change induced by post-spin strain depends on the spinning rate, but not on the post-spin strain rate.
- 3. The dependence of birefringence on spinning rate, on the extent of post-spin strain, and on post-spin strain rate can be described qualitatively in terms of a simple microstructural model. The relevant

microstructural parameters are the overall degree of molecular orientation and the extent of reinforcement by crystalline or NPL regions. A quantitative model is being developed.

- 4. Whereas increased birefringence correlates with increased tensile stiffness in conventional, synthetic polymer fibers, this is not the case for silk: birefringence increases with increasing silking rate between 0.5 cm s⁻¹ and at least 10 cm s⁻¹, but stiffness reaches a maximum within the same range of silking rate, at ~ 2 cm s⁻¹.
- 5. Processing silks and silk analogs economically for optimum fiber stiffness may not be achievable *via* conventional industrial routes of spinning *followed* by drawing. A single, slow-spinning step as used by nature would also seem uneconomic. Rapid spinning, followed by thermochemical treatment under load, may offer a necessary alternative.

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